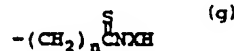
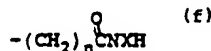
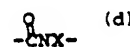
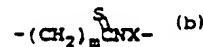
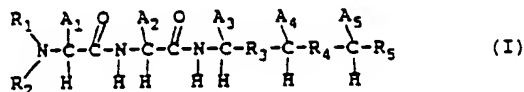


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(21) International Application Number: PCT/US93/10711 (22) International Filing Date: 4 November 1993 (04.11.93) (30) Priority data: 07/974,943 12 November 1992 (12.11.92) US (71) Applicant: BIOMEASURE, INC. [US/US]; 27 Maple Street, Milford, MA 01757 (US). (72) Inventors: KIM, Sun, Hyuk ; 20 Whitney Street, Chestnut Hill, MA 02167 (US). MOREAU, Jacques-Pierre ; 159 Westboro Road, Upton, MA 01568 (US). TAYLOR, John, E. ; 74 Fiske Mill Road, Upton, MA 01568 (US).		(74) Agent: TSAO, Y., Rocky; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US). (81) Designated States: AU, CA, CZ, FI, JP, NO, NZ, PL, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.

(54) Title: OPIOID PEPTIDES



(57) Abstract

Opioid peptides including those of formula (I), in which A₁ is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, and 2,6-dimethyltyrosine; A₂ is the identifying group of an amino acid selected from D-Ala and D-Arg; A₃ is H, or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine, A₄ is H, cyclohexylmethyl, the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, Phe, and substituted Phe with its benzene ring substituted by halogen, NO₂, OH, or CH₃; A₅ is the identifying group of a D- or L-amino acid selected from Leu, Nle, Lys, Met and Met(O), or is deleted together with R₄-CH attached thereto; each R₁ and R₂ is -H, -C(NH₂) = NH, or C₁₋₁₂ alkyl; R₃ is -(a) or (b); R₄ is (c); (d), or (e); and R₅ is -(CH₂)_{n+1}OH, (f), or (g); wherein m is 0-6, n is 0-6, and X is H, C₁₋₁₂ alkyl, C₆₋₁₂ aryl, C₇₋₁₈ aralkyl, C₇₋₁₈ alkaryl, C₆₋₁₇ pyridylalkyl, or C₆₋₁₇ alkylpyridyl; provided that when one of R₁ and R₂ is -C(NH₂) = NH, the other must be H; or a pharmaceutically acceptable salt thereof.

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OPIOID PEPTIDES

FIELD OF THE INVENTION

The present invention relates to short peptides.
5 More particularly, it relates to short peptides capable of selectively binding to receptors on cells.

BACKGROUND OF THE INVENTION

Since the discovery of endogenous opioid peptides in the 1970's, extensive research in opioid chemistry and
10 biology have suggested the existence of multiple opioid receptors: μ (mu), δ (delta) and κ (kappa).

The identification of multiple receptors is particularly interesting in that many opioids exert a variety of effects including analgesia, addiction,
15 respiratory depression, inhibition of gut transit, and cardiotoxicity. See e.g., *The Pharmacological Basis of Therapeutics*, McMillan, pp. 496-536, New York (1980); Hruby et al., *Med. Res. Rev.* 9:343 (1989); and Zimmermann, et al., *J. Med. Chem.* 33:895 (1990).

20 Recent work has suggested that opioid peptides may also be involved in pathological states, including cancer. As shown in Table 1, multiple opioid receptors are present on numerous tumor cell lines.

While the exact role played by opioid peptides in
25 oncogenic events remains unknown, opioids have been found to alter cell function and growth [Slotkin et al. *Life Sci.* 26:861 (1980); and Wilson et al. *J. Pharmacol. Exp. Ther.* 199:368 (1976)], to inhibit the growth of cultured neuroblastoma cells [Zagon et al. *Brain Res. Bull.* 7:25
30 (1981)], and to inhibit neuroblastoma tumor growth and prolong survival times, in an opioid antagonist sensitive manner, in mice with transplanted neuroblastomas, B-16 melanoma, MCF-7 breast cancer, human lung cancer cells

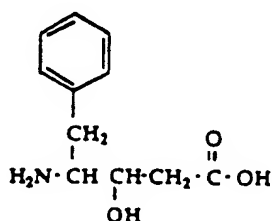
- 2 -

and others [Zagon et al. *Life Sci.* 28:1095 (1981); Zagon et al. *Science* 221:671 (1983); and Von Hoff et al. *Proc., Am. Assoc. Cancer Res.*, Abstract 932, p. 236 (1982); Srisuchark et al. *Int. J. Immunopharm.* 11(5):487 (1989);
 5 Minna et al. *Proc. Natl. Acad. Sci.*, 87:3294 (1990);
 ibid. 89:1169 (1992)].

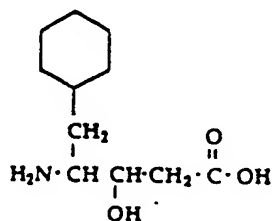
SUMMARY OF THE INVENTION

Structures and Abbreviations

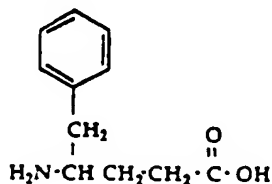
AHPPA = (3S,4S)-4-amino-3-hydroxy-5-phenyl-pentanoic acid



10 ACHPA = (3S,4S)-4-amino-5-cyclohexyl-3-hydroxy-pentanoic acid



APP = (4R)-4-amino-5-phenylpentanoic acid



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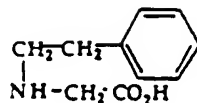
TABLE 1
Opiate Receptor Binding-Tumors or Tumor Cell Lines*

	<i>Tumor</i>	<i>Opiate Receptor Subtype</i>	<i>Receptor Conc. (fmol/mg protein)</i>
	Inventors' Data		
5	SCLC NCI-H69	mu	0
	SCLC NCI-H69	delta	234
	SCLC NCI-H69	kappa	0
	A549 NSCLC	mu	0
	A549 NSCLC	delta	0
10	A549 NSCLC	kappa	0
	MCF-7	mu	0
	MCF-7	delta	23
	MCF-7	kappa	0
	M 5123 Hepatoma	mu	0
15	B16 Melanoma	mu	0
	B16 Melanoma	kappa	129
	R3327 Prostate	mu	0
	<i>Data from Maneckjee et al. Proc. Natl. Acad. Sci. USA 87:3294 (1990)</i>		
	SCLC NCI-H187	nonselective	450
20	SCLC NCI-H69	nonselective	202
	SCLC NCI-H146	nonselective	172
	SCLC NCI-N417	nonselective	39
	SCLC NCI-H345	nonselective	18
	NSCLC NCI-H322	nonselective	293
25	NSCLC NCI-H460	nonselective	194
	NSCLC NCI-H157	nonselective	157
	NSCLC NCI-H23	nonselective	119
	NSCLC NCI-H290	nonselective	78
	<i>Data from Zagon et al J. Natl. Cancer Inst. 79:1059 (1987)</i>		
30	Breast Adenocarcinoma	delta	8.3
	Breast Adenocarcinoma	mu	14.2
	Breast Adenocarcinoma	kappa	10.0
	Ovarian Fibroma	delta	225.0
	Ovarian Fibroma	kappa	15.5
35	Endometrial Adenocarcinoma	delta	1.03
	Endometrial Adenocarcinoma	kappa	30.2
	Rectal Adenocarcinoma	delta	41.0
	Rectal Adenocarcinoma	kappa	54.0

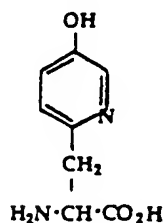
* does not exclude tumors expressing yet uncharacterized opiate receptor isotypes.

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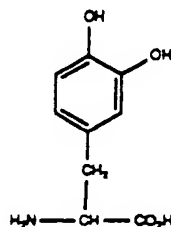
PEG = N-phenylethylglycine



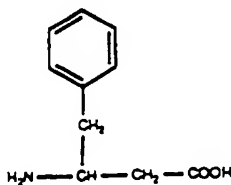
azatyrosine = L-3-(5-hydroxy-2-pyridyl)alanine



DOPA = 3,4-dihydroxyphenylalanine



homophenylalanine



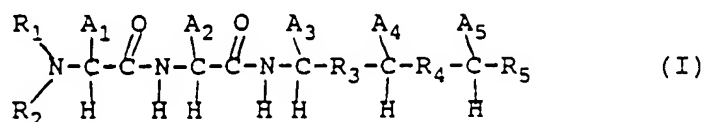
5 Nle = Norleucine

Met(O) = methionine sulfoxide

The present invention disclose a class of novel opioid peptides.

More specifically, one aspect of the invention
 10 relates to peptides of the formula:

- 5 -



in which

5 A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, and 2,6-dimethyltyrosine;

A_2 is the identifying group of an amino acid
10 selected from D-Ala and D-Arg;

A_3 is H, or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine,

A_4 is H, cyclohexylmethyl, the identifying group
15 of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, Phe, and substituted Phe with its benzene ring substituted by halogen, NO_2 , OH, or CH_3 ;

A_5 is the identifying group of a D- or L-amino
20 acid selected from Leu, Nle, Lys, Met and Met(O), or is deleted together with R_4-CH attached thereto;

each R_1 and R_2 is $-H$, $-C(NH_2)=NH$, or C_{1-12} alkyl;

R_3 is $-(CH_2)_m \overset{O}{\parallel} CNX-$ or $-(CH_2)_m \overset{S}{\parallel} CNX-$;

25 R_4 is $-CH(OH)CH_2 \overset{O}{\parallel} CNX-$; $-\overset{O}{\parallel} CNX-$, or $-\overset{S}{\parallel} CNX-$; and

R_5 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n \overset{O}{\parallel} CNXH$, or $-(CH_2)_n \overset{S}{\parallel} CNXH$ (m
30 is 0-6, n is 0-6, and X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{6-17} alkaryl, C_{6-17} alkyldiphenyl, or C_{6-17} alkylpyridyl); provided that when one of R_1 and R_2 is $-C(NH_2)=NH$, the other must be H.

Preferably, A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and
35 3,4-dimethoxyphenylalanine; A_5 is the identifying group of a D- or L-amino acid selected from Leu, Met and

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Met(O), or is deleted together with R₄-CH attached thereto; each R₁ and R₂

is -H or -C(NH₂)=NH; R₃ is -(CH₂)_m $\overset{\text{O}}{\parallel}$ CNX-; R₄ is -CH(OH)CH₂ $\overset{\text{O}}{\parallel}$ CNX-
 5 or $\overset{\text{O}}{\parallel}$ CNX-; R₅ is -(CH₂)_{n+1}OH or -(CH₂)_n $\overset{\text{O}}{\parallel}$ CNXH; m is 0-2; n is 0-2; and X is H, C₁₋₁₂ alkyl, or C₇₋₁₈ aralkyl.

Another aspect of the invention relates to peptides of formula (I), in which

10 A₁ is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-dimethyltyrosine;

A₂ is the identifying group of an amino acid
 15 selected from D-Ala and D-Arg;

A₃ is H or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

A₄ is H, cyclohexylmethyl, or the identifying
 20 group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

A₅ is the identifying group of a D- or L-amino acid selected from Leu, Nle, Lys, Met and Met(O), or is deleted together with R₄-CH attached thereto;

25 each R₁ and R₂ is -H, -C(NH₂)=NH, or C₁₋₁₂ alkyl;

R₃ is -(CH₂)_m $\overset{\text{O}}{\parallel}$ CNX- or -(CH₂)_m $\overset{\text{S}}{\parallel}$ CNX-;

R₄ is -CH(OH)CH₂ $\overset{\text{O}}{\parallel}$ CNX-; $\overset{\text{O}}{\parallel}$ CNX-, or $\overset{\text{S}}{\parallel}$ CNX-; and

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R_5 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n\overset{O}{\parallel}CNXH$, or $-(CH_2)_n\overset{S}{\parallel}CNXH$ (m is 0-6, n is 0-6, and X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl); provided that one and only one of A_3 and A_4 is H, and that when one of R_1 and R_2 is $-C(NH_2)=NH$, the other must be H.

Preferably, A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, and Tyr; A_5 is the identifying group of a D- or L-amino acid selected from Leu, Met and Met(O), or is deleted together with R_4-CH attached thereto; each R_1 and R_2 is $-H$ or $-C(NH_2)=NH$; R_3

is $-(CH_2)_m\overset{O}{\parallel}CNX-$; R_4 is $-CH(OH)CH_2\overset{O}{\parallel}CNX-$ or $-\overset{O}{\parallel}CNX-$; R_5 is $-(CH_2)_{n+1}OH$ or $-(CH_2)_n\overset{O}{\parallel}CNXH$; m is 0-2; n is 0-2; and X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.

A further aspect of the invention relates to peptides of formula (I), in which

A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-dimethyltyrosine;

A_2 is the identifying group of an amino acid selected from D-Ala and D-Arg;

A_3 is H or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

A_4 is the identifying group of an amino acid selected from Phe, and substituted Phe with its benzene ring substituted by halogen, NO_2 , OH, or CH_3 ;

A_5 is the identifying group of a D- or L-amino acid selected from Leu, Ile, Lys, Met and Met(O), or is deleted together with R_4-CH attached thereto;

each R_1 and R_2 is $-H$, $-C(NH_2)=NH$, or C_{1-12} alkyl;

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R_3 is $-(CH_2)_m \overset{\text{O}}{\parallel} CNX-$ or $-(CH_2)_m \overset{\text{S}}{\parallel} CNX-$;

R_4 is $-\text{CH}(\text{OH})\text{CH}_2 \overset{\text{O}}{\parallel} CNX-$; $-\overset{\text{O}}{\parallel} CNX-$, or $-\overset{\text{S}}{\parallel} CNX-$; and

5 R_5 is $-(CH_2)_{n+1}\text{OH}$, $-(CH_2)_n \overset{\text{O}}{\parallel} CNXH$, or $-(CH_2)_n \overset{\text{S}}{\parallel} CNXH$ (m is 1-6, n is 0-6, and X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl); provided that when
10 one of R_1 and R_2 is $-\text{C}(\text{NH}_2)=\text{NH}$, the other must be H.

Preferably, A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, and Tyr; A_5 is the identifying group of a D- or L-amino acid selected from Leu, Met and
15 Met(O), or is deleted together with R_4 -CH attached thereto; each R_1 and R_2 is $-\text{H}$ or $-\text{C}(\text{NH}_2)=\text{NH}$; R_3 is -

$(CH_2)_m \overset{\text{O}}{\parallel} CNX-$; R_4 is $-\text{CH}(\text{OH})\text{CH}_2 \overset{\text{O}}{\parallel} CNX-$ or $-\overset{\text{O}}{\parallel} CNX-$; R_5 is -
20 $(CH_2)_{n+1}\text{OH}$ or $-(CH_2)_n \overset{\text{O}}{\parallel} CNXH$; m is 1-2; n is 0-2; and X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.

The present invention also covers peptides of formula (I), in which

A_1 is the identifying group of an amino acid
25 selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-dimethyltyrosine;

A_2 is the identifying group of an amino acid selected from D-Ala and D-Arg;

30 A_3 is H or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

A_4 is H, cyclohexylmethyl, the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, Phe,
35

- 9 -

and substituted Phe with its benzene ring substituted by halogen, NO₂, OH, or CH₃;

A₅ is the identifying group of a D- or L-amino acid selected from Leu, Nle, Lys, Met and Met(O), or is
5 deleted together with R₄-CH attached thereto;

each R₁ and R₂ is -H, -C(NH₂)=NH, or C₁₋₁₂ alkyl;

R₃ is -CH₂NH- or -CO·NH-;

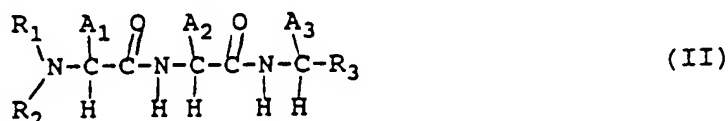
R₄ is -CH₂NH- or -CO·NH-; and

10 R₅ is -(CH₂)_{n+1}OH, -(CH₂)_n^OCNXH, or -(CH₂)_n^SCNXH (n is 0-6, and X is H, C₁₋₁₂ alkyl, C₆₋₁₂ aryl, C₇₋₁₈ aralkyl, C₇₋₁₈ alkaryl, C₇₋₁₈ alkaryl, C₆₋₁₇ pyridylalkyl, or C₆₋₁₇ alkylpyridyl); provided that one and only one of A₃ and A₄
15 must be H, and that one and only one of R₃ and R₄ is -CH₂NH-.

Preferably, A₁ is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, and Tyr; A₅ is the identifying
20 group of a D- or L-amino acid selected from Leu, Met and Met(O), or is deleted together with R₄-CH attached thereto; each R₁ and R₂ is -H or -C(NH₂)=NH; R₅ is -

(CH₂)_{n+1}OH or -(CH₂)_n^OCNXH; n is 0-2; and X is H, C₁₋₁₂
25 alkyl, or C₇₋₁₈ aralkyl. It is particularly preferred that A₄ be the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine.

Also within the invention are peptides of the
30 formula:



in which

35 A₁ is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-

- 10 -

dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-dimethyltyrosine;

A_2 is the identifying group of an amino acid selected from D-Ala and D-Arg;

5 A_3 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine, or is deleted together with CO-NH-CH attached thereto;

each R_1 and R_2 is -H, $-C(NH_2)=NH$, or C_{1-12} alkyl;

10 R_3 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n\overset{O}{\parallel}CNXH$, or $-(CH_2)_n\overset{S}{\parallel}CNXH$ (X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{7-18} 3,4-dihydroxyphenylalkyl, C_{7-18} 3,4-dimethoxyphenylalkyl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl).

15 Preferably, A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, and Tyr; each R_1 and R_2 is -H or

$-C(NH_2)=NH$; R_3 is $-(CH_2)_n\overset{O}{\parallel}CNXH$ (X is H, C_{1-12} alkyl, or C_{7-18} aralkyl). It is particularly preferred that A_3 be
20 deleted together with CO-NH-CH attached thereto.

Illustrative and non-limiting examples of peptides of the present invention are provided below:

DOPA-D-alanyl-glycyl-phenylalanyl-methionine amide,
25 DOPA-D-alanyl-glycyl-phenylalanyl methioninol,
DOPA-D-arginyl-glycyl-phenylalanyl-methionine amide,
DOPA-D-arginyl-glycyl-phenylalanyl methioninol,
3,4-dimethoxyphenylalanyl-D-alanyl-glycyl-phenylalanyl-
methionine amide,
30 3,4-dimethoxyphenylalanyl-D-alanyl-glycyl-phenylalanyl
methioninol,
3,4-dimethoxyphenylalanyl-D-arginyl-glycyl-phenylalanyl-
methionine amide,
3,4-dimethoxyphenylalanyl-D-arginyl-glycyl-phenylalanyl-
35 methioninol,
DOPA-D-alanyl-DOPA- β -alanine amide,

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- DOPA-D-arginyl-DOPA- β -alanine amide,
DOPA-D-alanyl-DOPA- β -alaninol,
DOPA-D-arginyl-DOPA- β -alaninol,
3,4-dimethoxyphenylalanyl-D-alanyl-DOPA- β -alanine amide,
5 3,4-dimethoxyphenylalanyl-D-arginyl-DOPA- β -alanine amide,
3,4-dimethoxyphenylalanyl-D-alanyl-DOPA- β -alaninol,
3,4-dimethoxyphenylalanyl-D-arginyl-DOPA- β -alaninol,
DOPA-D-alanyl-glycyl-PEG amide,
DOPA-D-arginyl-glycyl-PEG amide,
10 DOPA-D-alanyl-glycyl-APP amide,
DOPA-D-arginyl-glycyl-APP amide,
tyrosyl-D-alanyl-glycyl-PEG-methionine amide,
tyrosyl-D-arginal-glycyl-PEG-methionine amide,
tyrosyl-D-alanyl-glycyl-PEG methioninol,
15 tyrosyl-D-arginyl-glycyl-PEG methioninol,
tyrosyl-D-alanyl-glycyl-ACHPA amide,
tyrosyl-D-arginyl-glycyl-ACHPA amide,
tyrosyl-D-alanyl-glycyl-PEG amide,
tyrosyl-D-arginyl-glycyl-PEG amide,
20 tyrosyl-D-alanyl-DOPA-ACHPA amide,
tyrosyl-D-arginyl-DOPA-ACHPA amide,
tyrosyl-D-alanyl-DOPA-PEG amide,
tyrosyl-D-arginyl-DOPA-PEG amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-ACHPA amide,
25 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-ACHPA amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-PEG amide,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-PEG amide,
amidinotyrosyl-D-alanyl-glycyl-PEG amide,
amidinotyrosyl-D-arginyl-glycyl-PEG amide,
30 tyrosyl-D-alanyl-DOPA- β -alanine amide,
tyrosyl-D-arginyl-DOPA- β -alanine amide,
tyrosyl-D-alanyl-DOPA- β -alaninol,
tyrosyl-D-arginyl-DOPA- β -alaninol,
tyrosyl-D-alanyl-DOPA-glycinol,
35 tyrosyl-D-arginyl-DOPA-glycinol,

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- tyrosyl-D-alanyl-glycyl-DOPA amide,
tyrosyl-D-arginyl-glycyl-DOPA amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- β -alanine
amide,
5 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- β -alanine
amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- β -alaninol,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- β -alaninol,
tyrosyl-D-alanyl-glycyl-3,4-dimethoxyphenylalanyl amide,
10 tyrosyl-D-arginyl-glycyl-3,4-dimethoxyphenylalanyl amide,
tyrosyl-D-alanyl-glycyl-APP-methionine amide,
tyrosyl-D-alanyl-glycyl-APP methioninol,
tyrosyl-D-arginyl-glycyl-APP-methionine amide,
tyrosyl-D-arginyl-glycyl-APP methioninol,
15 tyrosyl-D-alanyl-glycyl-homophenylalanyl-methionine
amide,
tyrosyl-D-alanyl-glycyl-homophenylalanyl methioninol,
tyrosyl-D-arginyl-glycyl-homophenylalanyl-methionine
amide,
20 tyrosyl-D-arginyl-glycyl-homophenylalanyl methioninol,
tyrosyl-D-alanyl-glycyl-AHPPA amide,
tyrosyl-D-arginyl-glycyl-AHPPA amide,
tyrosyl-D-alanyl-glycyl-APP amide,
tyrosyl-D-arginyl-glycyl-APP amide,
25 tyrosyl-D-alanyl-glycyl-homophenylalanine amide,
tyrosyl-D-arginyl-glycyl-homophenylalanine amide,
tyrosyl-D-alanyl-DOPA-AHPPA amide
tyrosyl-D-arginyl-DOPA-AHPPA amide
tyrosyl-D-alanyl-DOPA-APP amide
30 tyrosyl-D-arginyl-DOPA-APP amide
tyrosyl-D-alanyl-DOPA-homophenylalanine amide,
tyrosyl-D-arginyl-DOPA-homophenylalanine amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-AHPPA amide,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-AHPPA amide,
35 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-APP amide,

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- tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-APP amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-
homophenylalanine amide,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-
5 homophenylalanine amide,
tyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
tyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
10 tyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
tyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
amidinotyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
15 ethylamide,
amidinotyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
amidinotyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
20 amidinotyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
tyrosyl-D-arginal-glycyl-phenylalanyl- ψ (CH₂NH)-leucine
amide,
tyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine ethylamide,
25 tyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine propylamide,
tyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine ethylamide,
tyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine
propylamide,
amidinotyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine
30 ethylamide,

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- amidinotyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine
propylamide,
amidinotyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine
ethylamide,
5 amidinotyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine
propylamide,
tyrosyl-D-arginal-DOPA-phenylalanyl- ψ (CH₂NH)-leucine
amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
10 phenylalanine ethylamide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine propylamide,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine ethylamide,
15 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine propylamide,
amidinotyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)- phenylalanine ethylamide,
amidinotyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-
20 ψ (CH₂NH)- phenylalanine propylamide,
amidinotyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)- phenylalanine ethylamide,
amidinotyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)- phenylalanine propylamide,
25 tyrosyl-D-arginal-3,4-dimethoxyphenylalanyl-phenylalanyl-
 ψ (CH₂NH)-leucine amide,
tyrosyl-D-alanyl-DOPA amide,
tyrosyl-D-arginyl-DOPA amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanine amide,
30 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanine amide,
tyrosyl-D-alanine 3-(3',4'-dihydroxyphenylpropyl)amide,
tyrosyl-D-arginine 3-(3',4'-dihydroxyphenylpropyl)amide,
tyrosyl-D-alanine 3-(3',4'-dimethoxyphenylpropyl)amide,
tyrosyl-D-arginine 3-(3',4'-dimethoxyphenylpropyl)amide,
35 DOPA-D-alanine 3-phenylpropylamide,

- 15 -

DOPA-D-alanine 2-(2-aminoethylpyridyl)amide, and
DOPA-D-arginine 2-(2-aminoethylpyridyl)amide.

In this disclosure, the identifying group of an α -
amino acid is the atom or group of atoms bound to the
5 asymmetric α -carbon atom, other than the carbonyl carbon
atom, the amino nitrogen atom and the H atom. To
illustrate by examples, the identifying group of alanine
is $-\text{CH}_3$ and the identifying group of phenylalanine is
(C_6H_5) CH_2- .

10 Also, unless specified as an L- or D-isomer, an
amino acid is intended to be an L amino acid.

Further, a short line between two amino acid
residues (e.g., DOPA-D-alanine 3-phenylpropylamide)
represents a peptide bond; that is, a covalent bond
15 between C of a carbonyl group and N of an amino group.
The symbol ψ indicates the presence of a non-peptide bond
and the nature of the non-peptide bond is described in
the parentheses following ψ (e.g., tyrosyl-D-alanyl-
glycyl- $\psi(\text{CH}_2\text{NH})$ -phenylalanine ethylamide). Thus, -
20 $\psi(\text{CH}_2\text{NH})-$ represents the presence of a bond between two
amino acid residues in which the carbon atom
participating in the bond is reduced from a carbonyl
carbon to a methylene carbon.

A detailed discussion of the chemistry of non-
25 peptide bonds is given in Coy et al. *Tetrahedron* 44:835
(1988); Tourwe Janssen *Chim. Acta* 3:3-15, 17-18 (1985);
and Spatola in *Chemistry and Biochemistry of Amino Acids,*
Peptides and Proteins (B. Weinstein, ed.), M. Dekker, New
York and Basal, pp. 267-357 (1983). All of them are
30 hereby incorporated by reference.

Pharmaceutically acceptable salts of the above-
described peptides are also within the present invention.
Examples of preferred salts include those formed with
therapeutically acceptable acids, e.g., hydrochloric,
35 hydrobromic, sulfuric, nitric, phosphoric, citric,

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acetic, maleic, lactic, malic, ascorbic succinic,
benzoic, fumaric, salicylic, methanesulfonic,
trifluoroacetic, toluenesulfonic, or pamoic acid, as well
as polymeric acids such as tannic acid or carboxymethyl
5 cellulose, lactate/glycolate.

Opioid peptides are known to possess analgesic,
antitussive, and antidiarrheal activity and thus may be
used in human or veterinary medicine for the relief or
prevention of pain, for the treatment of diarrhea or
10 dysentery, for the suppression of cough and for
hypertension. Further, the opioid peptides of the
invention are effective in treating various cancers
(e.g., lung, breast, melanoma, or neuroblastoma).

A therapeutically effective amount of a peptide of
15 the invention and a pharmaceutically acceptable carrier
substance (e.g., magnesium carbonate or lactose) can be
formulated to form a therapeutic composition, such as (i)
a pill, tablet, capsule, or liquid for oral
administration to a patient; (ii) a liquid or an ointment
20 capable of being administered by inhalation,
transdermally, nasally, rectally or sublingually; (iii) a
liquid capable of being administered intravenously,
parenterally, subcutaneously or intraperitoneally; or
(iv) an oral or a parenteral sustained release
25 formulation. Thus, the opioid peptide of the invention
may be administered to a mammal, particularly a human, in
one of the traditional modes (e.g., orally, parenterally,
transdermally, or transmucosally), in a sustained release
formulation using a biodegradable biocompatible polymer,
30 or by on-site delivery using micelles, gels and
liposomes. The peptides can be administered to a human
patient in a dosage of 1000 $\mu\text{g/kg/day}$ to 50 mg/kg/day .

The peptides of the present invention can also be
used as tools for detecting specific opioid receptors in
35 cells of certain tissues or organs.

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Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 Synthesis

The peptides of this invention can be readily prepared by standard solution or solid phase peptide synthesis. Thus, procedures analogous to those disclosed in *Solid Phase Peptide Synthesis* by Stewart and Young,
10 Pierce Co., Illinois, 1984 or *Principles of Peptide Synthesis* by Springer-Verlag, Berlin, 1984 may be followed. Both of them are hereby incorporated by reference.

Peptides of the invention that contain a CH_2NH
15 non-peptide bond can be prepared by reacting on N-protected amino acid aldehyde with the free amino group of another C-protected amino acid using NaCNBH_3 , as described in Martinez et al. *J. Med. Chem.* 28:1874 (1985) and Coy et al. *Tetrahedron* 44:835 (1988), both of which
20 are hereby incorporated by reference.

AHPPA and ACHPA can be synthesized according to the method of Hui et al. *J. Med. Chem.* 30:1281 (1987); Schuda et al., 1987, *J. Org. Chem.* 53:873; and Rich et al., 1988, *J. Org. Chem.* 53:869. All three of them are
25 hereby incorporated by reference.

To obtain N-terminal amidinotyrosyl peptides, the peptide salt (e.g., an acetates or trifluoroacetate salt) was reacted with 3,5-dimethylpyrazolo-1-carboxamide nitrate in the presence of base, e.g. triethylamine,
30 diisopropylamine, or aqueous NaOH (pH 9.3-9.5), in inert solvents, e.g. alcohol, water, tetrahydrofuran or its mixture, at 0-80°C, 2 hours to several days. The intermediates and final products were isolated and purified by standard methods, e.g., by column

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chromatography, crystallization on high performance liquid chromatography ("HPLC"). Purity was determined using chromatographic, spectroscopic and chemical analysis.

- 5 Benzyloxycarbonyl ("CBZ")-tyrosyl-D-alanyl-glycyl-PEG glycine amide was synthesized as follows. 2.3 ml diisopropylamine was added to a mixture of CBZ-tyrosyl-D-alanine (1.65 g), glycyl-PEG amide trifluoroacetic acid salt (1.5 g) and BOP reagent (i.e.,
- 10 benzotriazol-1-yloxytris (dimethylamine)phosphonium hexafluorophosphate) (2.1 g) in 20 ml dimethylformamide ("DMF") and the mixture was stirred at room temperature overnight. Solvent was removed in vacuo to a dryness and the residue partitioned between ethylacetate and water.
- 15 The organic layer and any partially insoluble solids were combined, the solvent evaporated, and the residue recrystallized from ethylacetate. Yield: 0.65 g; Thin layer chromatography ("TLC") (Silica gel: $\text{CHCl}_3/\text{MeOH}$ 9:1, R_f 0.37).
- 20 CBZ-tyrosyl-D-alanyl-glycyl-PEG amide (0.6g) in 20 ml methanol was hydrogenated under 32 psi using 100 mg 10% Pd-C for 4 hours. The mixture was then filtered through celite pad, washed with alcohol. Thereafter, the filtrate was concentrated in vacuo to dryness. Yield:
- 25 0.49 g colorless solid; TLC (silica gel; $\text{CHCl}_3/\text{MeOH}$ =4:1, R_f 0.1). Synthetic amino acids are commercially available.

- (4R)-Boc-amino-5-phenyl-pentanoic acid was synthesized as follows. To a partial solution of Boc-
- 30 phenylalanine adehyde (2.3 g) in 30 ml dichloromethane cooled to 0-5°C, was added carbethoxymethylene triphenylphosphorane (10 g). The mixture was stirred at 0-5°C for 3 hours then room temperature overnight. Solvent was evaporated in vacuo to a dryness and the
- 35 residue was triturated with boiling ether (~200 ml).

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Ether extract was concentrated in vacuo and the residue was chromatographed on silica gel (50 g) using hexane/ethylacetate (5:1) as eluants. Appropriate fractions were pooled and solvents removed in vacuo to a dryness. Yield: 2.1 g; TLC (Silica gel: hexane/ethylacetate = 2:1, Rf 0.57). The product was then dissolved in 20 μ l EtOH to which 150 mg of 10% Pd-C was added. Hydrogenation was carried out under 30 psi for 5½ hours. The mixture was filtered through celite pad, washed with alcohol and the filtrate was concentrated in vacuo to a dryness. Yield: 2.1 g colorless solid. 1.4 g of the colorless solid was suspended in 20 ml methanol and, upon addition of 6 ml 2N-NaOH, stirred at room temperature for 1 hour. After evaporation of solvent, aqueous layer was acidified to pH 2-3, extracted with ethylacetate, and dried (MgSO₄). Solvent was evaporated in vacuo to a dryness. Yield: 1.23 g colorless solid; TLC (Silica gel: CHCl₃/MeOH/HoAc 9:1:0.1, Rf 0.62).

20 Tyrosyl-D-alanyl-glycyl-AHPPA amide was synthesized as follows. Boc-AHPPA was first incorporated on 4-methylbenzhydrylamine resin. 1.0 g (0.64 mmole) 4-methylbenzhydrylamine-polystyrene resin (Bachem, Inc.) in the chloride ion form was placed in the reaction vessel of an Advanced ChemTech 200 peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 10% triethylamine in chloroform; (c) methylene chloride; and (d) dimethylformamide. The neutralized resin was mixed for 18 hours with the preformed active ester made from Boc AHPPA (1.92 mmole), diisopropyl carbodiimide (1.92 mmole), and hydroxybenzotriazole hydrate (1.92 mmole) in dimethylformamide in an ice bath for 1 hour. The resulting amino acid resin was washed on the synthesizer with dimethylformamide followed by methylene chloride.

- 20 -

Acetylation of any free amino group of the resin was performed by mixing the amino acid resin for 15 minutes with N-acetyl imidazole (5 mmole) in methylene chloride.

The remaining amino acids were coupled as follows.

- 5 The peptide synthesizer was programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid ("TFA") in methylene chloride 2 times (5 min. and 25 min. each); (c) methylene chloride; (d) isopropyl alcohol; (e) 10% triethylamine in
10 chloroform; and (f) methylene chloride. The following amino acid (e.g. Boc-glycine) and diisopropyl carbodiimide (3 eq. each) in methylene chloride were mixed for 2 hours and the resulting amino acid resin was then cycled through steps (a) to (f) in the above
15 procedure. The next following Boc-amino acids (Boc-D-alanine, Boc-tyrosine) (3 eq. each) were coupled successively following the same procedure. After drying, the resin (1.3 g) was mixed with anisole (5 ml), dithiothreitol (200 mg) and anhydrous hydrogen fluoride
20 (35 ml) at 0°C for 45 minutes. Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen and the free peptide was precipitated and washed with ether. The crude peptide was then dissolved in a minimum volume of 1 M acetic acid and applied to Vydac C18 column (2.54
25 cm I.D. x 35 cm). The peptide was eluted with a gradient (20%-80%) of 50/50 0.1% trifluoroacetic acid/acetonitrile in 0.1% trifluoroacetic acid in water. Fractions were examined by analytical HPLC. Most of the solvent was removed in vacuo to yield a small volume, and thereafter
30 the product lyophilized. Yield: 24 mg colorless solid.

Tyrosyl-D-arginyl-glycyl-phenylalanyl- ψ (CH₂NH)-leucine amide was synthesized as follows. The procedure is essentially as described above except for the following reductive alkylation step. Boc-phenylalanine
35 aldehyde (2.5 eq), prepared by the method of Pehrentz and

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Castro, *Synthesis*, p. 676 (1983), hereby incorporated by reference, was first dissolved in 5 ml of dry DMF and then added to the suspension of TFA salt of leucine resin in DMF containing 1% acetic acid, followed by the
 5 portion-wise addition of sodium cyanoborohydride (4 eq) over 40 minutes. After stirring for 1 hour, the resin mixture was found to be negative to ninhydrin reaction.

Other compounds can be prepared in one or more of the manners described above and screened for
 10 effectiveness following procedures set forth below. Both the synthetic and screening methods are well known to a person of ordinary skill in the art.

Activity

(1) In vitro inhibition of radioligand to opioid
 15 receptors

The ability of various opioid peptides of the present invention to selectively inhibit binding of μ receptor ligand to μ receptor is shown in Table 2.

20

TABLE 2
IN VITRO RECEPTOR BINDING-K_i (nM)

CODE	μ RECEPTOR DAGO	δ RECEPTOR DPPE	κ RECEPTOR U69,593
BIM-38052	0.19 \pm 0.03	27.17 \pm 3.23	241.67 \pm 10.93
25 BIM-38031	0.65 \pm 0.05	12.60 \pm 2.52	205.00
BIM-38020	1.18 \pm 0.09	1014.00 \pm 420.00	319.75 \pm 9.92
BIM-38007	1.36 \pm 0.28	129.67 \pm 108.72	524.67 \pm 23.25
BIM-38039	1.48 \pm 0.24	297.00 \pm 52.60	3763.33 \pm 728.28
BIM-38012	2.57 \pm 0.30	540.00 \pm 432.24	369.00 \pm 42.75
30 BIM-38009	2.92 \pm 0.69	14482.00	3258.33 \pm 165.30
BIM-38046	2.92 \pm 1.12	72.65 \pm 2.59	849.00
BIM-38040	3.97 \pm 0.67	1440.33 \pm 368.08	1413.67 \pm 201.26
BIM-38013	4.01 \pm 0.48	161.50 \pm 450	192.00 \pm 27.71
BIM-38026	4.27 \pm 0.46	225.00 \pm 72.00	
35 2755.00 \pm 2246.00			
BIM-38023	4,73 \pm 1.55	413.50 \pm 215.50	
5201.33 \pm 2241.98			

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The following procedure was used to determine the ability of various opioid peptides to inhibit binding at opioid receptors. Crude membranes were prepared by

5 homogenization of guinea-pig or rat forebrain samples in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (setting 6, 15 sec.). Buffer was added to obtain a final volume of 40 ml, and the homogenate centrifuged in a Sorval SS-34 rotor at 39,000 g for 10 min. at 0-4°C. The

10 resulting supernatant was decanted and discarded. The pellet was rehomogenized in ice-cold buffer, pre-incubated at 37°C for 45 min., diluted, and centrifuged as before. The final pellet was resuspended in 50 mM Tris HCl and held on ice for the receptor binding assay.

15 Aliquots of the washed membrane preparation (described above) were incubated in 50 mM Tris HCl at a total volume of 2.0 ml at 25°C with an opioid receptor ligand (see below) and various concentrations of the unlabeled test compounds. Mu and kappa receptor assays

20 were incubated for 60 minutes. Delta receptor assays were incubated for 40 minutes. At the end of the incubation periods, the assays were terminated by rapid filtration through Whatman GF/B glass fiber filters, and the bound radioactivity trapped on the filters counted by

25 liquid scintillation spectrometry. For each of the three receptor subtypes, specific binding was calculated as the total radioligand bound minus that bound in the presence of 1000 nM levallorphan.

Three radiolabeled opioid receptor ligands, DAGO,

30 DPPE, and U69,593, were used in μ receptor, δ receptor and κ receptor assays, respectively. The structures of the ligands are as follows.

DAGO: Tyr-D-Met-Gly-Me-Phe-NH-CH₂-CH₂-OH;

DPPE: Tyr-D-Penicilliamine-Gly-Phe-D-Penicilliamine

35 (cyclized); and

- 23 -

U69,953: (5,7,8)-(-)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)dec-8-yl)-benzeneacetamide.

Inhibition constants ("Ki") were calculated from the equation $K_i = IC_{50} / (1 + L/K_d)$, where L is the
5 radioligand concentration and K_d is the equilibrium dissociation constant for the radioligand. The IC_{50} was derived from the inhibition data by linear least squares regression of $\log (B/B_t - B)$ versus $\log I$, where I is the
10 test compound concentration, B_t is the total amount of radioligand specifically bound, and B is the amount of specific binding in the presence of a given concentration of the unlabeled peptide. The IC_{50} is the antilog of $\log I$, when the expression, $\log (B/B_t - B)$ equals zero.

The structures of the test compounds are as
15 follows.

- BIM-38052: tyrosyl-D-arginyl-glycyl-APP amide
- BIM-38031: tyrosyl-D-alanyl-glycyl-APP amide
- BIM-38020: tyrosyl-D-arginyl-glycyl-PEG amide
- BIM-38007: tyrosyl-D-alanyl-glycyl-AHPPA amide
- 20 BIM-38039: tyrosyl-D-alanyl-glycyl-PEG-methionine amide
- BIM-38012: tyrosyl-D-alanyl-glycyl-ACHPA amide
- BIM-38009: tyrosyl-D-alanyl-glycyl-PEG amide
- BIM-38046: tyrosyl-D-alanyl-glycyl-homophenylalanine
amide
- 25 BIM-38040: tyrosyl-D-alanyl-glycyl-PEG methioninol
- BIM-38013: tyrosyl-D-arginyl-glycyl-phenylalanyl-
 ψ -(CH₂NH)-leucine amide
- BIM-38026: tyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide
- 30 BIM-38023: tyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide

(2) Sodium ions discriminate opioid agonist/antagonist
properties

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NaCl decreases the potency of opioid agonists (i.e., morphine) and has no effect on the potency of opioid antagonists (i.e., naloxone). Several tested opioid peptides of the invention all showed some degree of agonist property, as evidenced by a decreased potency in the presence of NaCl. See Table 3 below.

TABLE 3
The Effect of Sodium Ion on the Binding to
Mu Opiate Receptors In Vitro
Ki (nM)

10

	-NaCl	+NaCl*	-NaCl/+NaCl
BIM-38007	4.0±0.6(3)	13±4.0(2)	3.3
15 BIM-38009	6.9±3.6(3)	68±8.5(2)	9.9
BIM-38005	19±3.6(5)	145±14(3)	7.6
Morphine	3.6	140	39
[D-Ala ² , D-Leu ³] enkephalin	15	560	37
20 naloxone	0.9	0.7	0.8

* 100 mM

The binding experiments described in Table 3 were performed as described above, except for the presence of 100 mM NaCl.

25

- 25 -

(3) *In Vivo* Growth Inhibition of Melanomas by Opioid Peptide

As shown in Table 4, administration of two opioid peptides (BIM-38007 and BIM-38009, see above for structures) inhibited the growth of B16-F10 melanomas in *in vivo* animal experiments. In these experiments, tumors were induced in BALB/c athymic nude mice. 50 μ g of peptide in 0.2 ml of saline was injected subcutaneously twice a day on days 1-5, 6-days Subrenal Capsule Assay ("SRCA").

Control animals received 0.2 ml of saline subcutaneously twice daily on days 1-5. Mice were sacrificed on day 6 and tumor size determined by a microscope. The results are reported as the change in tumor size from day 0 (time of tumor implantation) to day 6 (time of assay termination).

TABLE 4
Effect of Opioid Peptides on Tumor Size

B-16 Melanoma

20

Group No.	Treatment	Change In Tumor Size (omu)	% T/C
25	1 Vehicle Treated Control	21.3 \pm 3.3	--
	2 BIM-38007	18.1 \pm 2.0	85
	3 BIM-38009	14.7 \pm 3.1	69

omu=ocular micrometer unit, 1.0 mm=10 omu; % T/C=omu experimental/omu control

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Table 5 compares the antitumor activity of an opioid BIM-38009 versus methadone. A subcutaneous tumor assay was used in which xenografts of the human non-small cell lung cancer were implanted s.c. in athymic nude mice. Test compounds were administered s.c. or i.p., twice daily, from day 4 post tumor implantation to day 35. The results are reported as means \pm s.e.m. on 8 animals per group. For structure of BIM-38009, see above.

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TABLE 5
Antitumor Activity of BIM-38009: Human NSLC A549

5	Group No.	Treatment	Tumor Weight(mg) (Day 34)	% T/C
10	1	Saline Vehicle Treated control, 0.2 ml/inj., s.c., b.i.d., q.d. 4-35	305±60	--
	2*	BIM-38009, 500 µg/inj., s.c., b.i.d., q.d., 4-35	134±36	44
15	3	Methadone, 10 mg/kg/inj., i.p., q.d. 4-35	337±57	110

* Significance of difference from control: $p < 0.01$

OTHER EMBODIMENTS

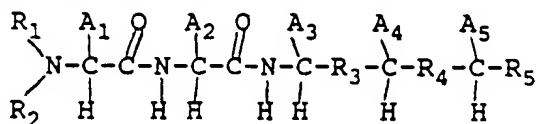
20 The foregoing description has been limited to
specific embodiments of this invention. It will be
apparent, however, that variations and modifications may
be made to the invention, with the attainment of some or
all of the advantages of the invention. Such embodiments
25 are also within the scope of the following claims.

What is claimed is:

- 28 -

Claims

1. A peptide of the formula:



in which

- 5 A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, and 2,6-dimethyltyrosine;

- 10 A_2 is the identifying group of an amino acid selected from D-Ala and D-Arg;

A_3 is H, or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine,

- 15 A_4 is H, cyclohexylmethyl, the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, Phe, and substituted Phe with its benzene ring substituted by halogen, NO_2 , OH, or CH_3 ;

- 20 A_5 is the identifying group of a D- or L-amino acid selected from Leu, Nle, Lys, Met and Met(O), or is deleted together with R_4-CH attached thereto;

each R_1 and R_2 is -H, $-C(NH_2)=NH$, or C_{1-12} alkyl;

R_3 is $-(CH_2)_m \overset{O}{\parallel} CNX-$ or $-(CH_2)_m \overset{S}{\parallel} CNX-$;

- 25 R_4 is $-CH(OH)CH_2 \overset{O}{\parallel} CNX-$; $-\overset{O}{\parallel} CNX-$, or $-\overset{S}{\parallel} CNX-$; and

R_5 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n \overset{O}{\parallel} CNXH$, or $-(CH_2)_n \overset{S}{\parallel} CNXH$;

- 30 wherein m is 0-6, n is 0-6, and X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{7-18} alkaryl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl; provided that when one of R_1 and R_2 is $-C(NH_2)=NH$, the other must be H; or a pharmaceutically acceptable salt thereof.

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2. The peptide of claim 1, wherein

A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

5 A_5 is the identifying group of a D- or L-amino acid selected from Leu, Met and Met(O), or is deleted together with R_4 -CH attached thereto;

each R_1 and R_2 is -H or $-C(NH_2)=NH$;

10 R_3 is $-(CH_2)_m \overset{\text{O}}{\parallel} CNX-$;

R_4 is $-CH(OH)CH_2 \overset{\text{O}}{\parallel} CNX-$ or $-\overset{\text{O}}{\parallel} CNX-$;

R_5 is $-(CH_2)_{n+1}OH$ or $-(CH_2)_n \overset{\text{O}}{\parallel} CNXH$;

15 m is 0-2;

n is 0-2; and

X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.

3. The peptide of claim 2 having the formula of:

DOPA-D-alanyl-glycyl-phenylalanyl-methionine amide,

20 DOPA-D-alanyl-glycyl-phenylalanyl methioninol,

DOPA-D-arginyl-glycyl-phenylalanyl-methionine amide,

DOPA-D-arginyl-glycyl-phenylalanyl methioninol,

3,4-dimethoxyphenylalanyl-D-alanyl-glycyl-phenylalanyl-methionine amide,

25 3,4-dimethoxyphenylalanyl-D-alanyl-glycyl-phenylalanyl-methioninol,

3,4-dimethoxyphenylalanyl-D-arginyl-glycyl-phenylalanyl-methionine amide,

3,4-dimethoxyphenylalanyl-D-arginyl-glycyl-phenylalanyl-methioninol,

30

DOPA-D-alanyl-DOPA- β -alanine amide,

DOPA-D-arginyl-DOPA- β -alanine amide,

DOPA-D-alanyl-DOPA- β -alaninol,

DOPA-D-arginyl-DOPA- β -alaninol,

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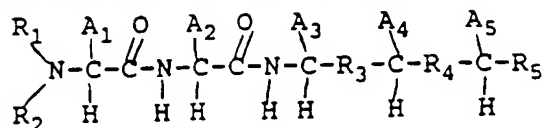
- 3,4-dimethoxyphenylalanyl-D-alanyl-DOPA- β -alanine amide,
3,4-dimethoxyphenylalanyl-D-arginyl-DOPA- β -alanine amide,
3,4-dimethoxyphenylalanyl-D-alanyl-DOPA- β -alaninol,
3,4-dimethoxyphenylalanyl-D-arginyl-DOPA- β -alaninol,
5 DOPA-D-alanyl-glycyl-PEG amide,
DOPA-D-arginyl-glycyl-PEG amide,
DOPA-D-alanyl-glycyl-APP amide, or
DOPA-D-arginyl-glycyl-APP amide.

4. The peptide of claim 3 having the formula of:

- 10 DOPA-D-alanyl-glycyl-phenylalanyl-methionine amide,
DOPA-D-arginyl-glycyl-phenylalanyl-methionine amide,
3,4-dimethoxyphenylalanyl-D-alanyl-glycyl-phenylalanyl-
methionine amide,
3,4-dimethoxyphenylalanyl-D-arginyl-glycyl-phenylalanyl-
15 methionine amide,
DOPA-D-alanyl-DOPA- β -alanine amide,
DOPA-D-arginyl-DOPA- β -alanine amide,
3,4-dimethoxyphenylalanyl-D-alanyl-DOPA- β -alanine amide,
3,4-dimethoxyphenylalanyl-D-arginyl-DOPA- β -alanine amide,
20 DOPA-D-alanyl-glycyl-PEG amide,
DOPA-D-arginyl-glycyl-PEG amide,
DOPA-D-alanyl-glycyl-APP amide, or
DOPA-D-arginyl-glycyl-APP amide.

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5. A peptide of the formula:



5 in which

A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-dimethyltyrosine;

10 A_2 is the identifying group of an amino acid selected from D-Ala and D-Arg;

A_3 is H or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

15 A_4 is H, cyclohexylmethyl, or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

A_5 is the identifying group of a D- or L-amino acid selected from Leu, Nle, Lys, Met and Met(O), or is
20 deleted together with R_4 -CH attached thereto;

each R_1 and R_2 is -H, $-C(NH_2)=NH$, or C_{1-12} alkyl;

R_3 is $-(CH_2)_m \overset{O}{\parallel} CNX-$ or $-(CH_2)_m \overset{S}{\parallel} CNX-$;

25 R_4 is $-CH(OH)CH_2 \overset{O}{\parallel} CNX-$; $-\overset{O}{\parallel} CNX-$, or $-\overset{S}{\parallel} CNX-$;

R_5 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n \overset{O}{\parallel} CNXH$, or $-(CH_2)_n \overset{S}{\parallel} CNXH$;

wherein m is 0-6, n is 0-6, and X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl; provided that one and
30 only one of A_3 and A_4 is H, and that when one of R_1 and R_2 is $-C(NH_2)=NH$, the other must be H; or a pharmaceutically acceptable salt thereof.

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6. The peptide of claim 5, wherein

A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, and Tyr;

5 A_5 is the identifying group of a D- or L-amino acid selected from Leu, Met and Met(O), or is deleted together with R_4 -CH attached thereto;

each R_1 and R_2 is -H or $-C(NH_2)=NH$;

10 R_3 is $-(CH_2)_m \overset{\text{O}}{\parallel} CNX-$;

R_4 is $-CH(OH)CH_2 \overset{\text{O}}{\parallel} CNX-$ or $-\overset{\text{O}}{\parallel} CNX-$;

R_5 is $-(CH_2)_{n+1}OH$ or $-(CH_2)_n \overset{\text{O}}{\parallel} CNXH$;

15 m is 0-2;

n is 0-2; and

X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.

7. The peptide of claim 6 having the formula of:

tyrosyl-D-alanyl-glycyl-PEG-methionine amide,
 20 tyrosyl-D-arginal-glycyl-PEG-methionine amide,
 tyrosyl-D-alanyl-glycyl-PEG methioninol,
 tyrosyl-D-arginyl-glycyl-PEG methioninol,
 tyrosyl-D-alanyl-glycyl-ACHPA amide,
 tyrosyl-D-arginyl-glycyl-ACHPA amide,
 25 tyrosyl-D-alanyl-glycyl-PEG amide,
 tyrosyl-D-arginyl-glycyl-PEG amide,
 tyrosyl-D-alanyl-DOPA-ACHPA amide,
 tyrosyl-D-arginyl-DOPA-ACHPA amide,
 tyrosyl-D-alanyl-DOPA-PEG amide,
 30 tyrosyl-D-arginyl-DOPA-PEG amide,
 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-ACHPA amide,
 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-ACHPA amide,
 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-PEG amide,
 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-PEG amide,
 35 amidinotyrosyl-D-alanyl-glycyl-PEG amide,

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- amidinotyrosyl-D-arginyl-glycyl-PEG amide,
 tyrosyl-D-alanyl-DOPA- β -alanine amide,
 tyrosyl-D-arginyl-DOPA- β -alanine amide,
 tyrosyl-D-alanyl-DOPA- β -alaninol,
 5 tyrosyl-D-arginyl-DOPA- β -alaninol,
 tyrosyl-D-alanyl-glycyl-DOPA amide,
 tyrosyl-D-arginyl-glycyl-DOPA amide,
 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- β -alanine
 amide,
 10 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- β -alanine
 amide,
 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- β -alaninol,
 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- β -alaninol,
 tyrosyl-D-alanyl-glycyl-3,4-dimethoxyphenylalanyl amide,
 15 or
 tyrosyl-D-arginyl-glycyl-3,4-dimethoxyphenylalanyl amide.

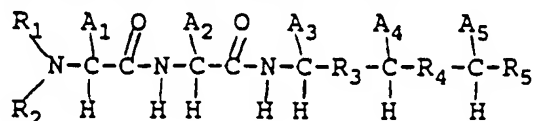
8. The peptide of claim 7 having the formula of:
 tyrosyl-D-alanyl-glycyl-PEG-methionine amide,
 tyrosyl-D-arginal-glycyl-PEG-methionine amide,
 20 tyrosyl-D-alanyl-glycyl-PEG methioninol,
 tyrosyl-D-arginyl-glycyl-PEG methioninol,
 tyrosyl-D-alanyl-glycyl-ACHPA amide,
 tyrosyl-D-arginyl-glycyl-ACHPA amide,
 tyrosyl-D-alanyl-glycyl-PEG amide,
 25 tyrosyl-D-arginyl-glycyl-PEG amide,
 amidinotyrosyl-D-alanyl-glycyl-PEG amide,
 amidinotyrosyl-D-arginyl-glycyl-PEG amide,
 tyrosyl-D-alanyl-DOPA- β -alanine amide,
 tyrosyl-D-arginyl-DOPA- β -alanine amide,
 30 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- β -alanine
 amide,
 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- β -alanine
 amide,

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tyrosyl-D-alanyl-glycyl-3,4-dimethoxyphenylalanyl amide,
or
tyrosyl-D-arginyl-glycyl-3,4-dimethoxyphenylalanyl amide.

9. A peptide of the formula:

5



in which

A_1 is the identifying group of an amino acid
10 selected from 3,4-dihydroxyphenylalanine, 3,4-
dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-
dimethyltyrosine;

A_2 is the identifying group of an amino acid
selected from D-Ala and D-Arg;

15 A_3 is H or the identifying group of an amino acid
selected from 3,4-dihydroxyphenylalanine and 3,4-
dimethoxyphenylalanine;

A_4 is the identifying group of an amino acid
selected from Phe, and substituted Phe with its benzene
20 ring substituted by halogen, NO_2 , OH, or CH_3 ;

A_5 is the identifying group of a D- or L-amino
acid selected from Leu, Nle, Lys, Met and Met(O), or is
deleted together with R_4-CH attached thereto;

each R_1 and R_2 is -H, $-C(NH_2)=NH$, or C_{1-12} alkyl;

25

R_3 is $-(CH_2)_m \overset{O}{\parallel} CNX-$ or $-(CH_2)_m \overset{S}{\parallel} CNX-$;

R_4 is $-CH(OH)CH_2 \overset{O}{\parallel} CNX-$; $-\overset{O}{\parallel} CNX-$, or $-\overset{S}{\parallel} CNX-$;

30

R_5 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n \overset{O}{\parallel} CNXH$, or $-(CH_2)_n \overset{S}{\parallel} CNXH$;

wherein m is 1-6, n is 0-6, and X is H, C_{1-12}
alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{7-18}
alkaryl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl;
provided that when one of R_1 and R_2 is $-C(NH_2)=NH$, the

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other must be H; or a pharmaceutically acceptable salt thereof.

10. The peptide of claim 9, wherein

5 A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, and Tyr;

A_5 is the identifying group of a D- or L-amino acid selected from Leu, Met and Met(O), or is deleted together with R_4 -CH attached thereto;

10 each R_1 and R_2 is -H or $-C(NH_2)=NH$;

R_3 is $-(CH_2)_m \overset{\text{O}}{\parallel} CNX-$;

R_4 is $-CH(OH)CH_2 \overset{\text{O}}{\parallel} CNX-$ or $-\overset{\text{O}}{\parallel} CNX-$;

15 R_5 is $-(CH_2)_{n+1}OH$ or $-(CH_2)_n \overset{\text{O}}{\parallel} CNXH$;

m is 1-2;

n is 0-2; and

X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.

20 11. The peptide of claim 10 having the formula of:

tyrosyl-D-alanyl-glycyl-APP-methionine amide,

tyrosyl-D-alanyl-glycyl-APP methioninol,

tyrosyl-D-arginyl-glycyl-APP-methionine amide,

25 tyrosyl-D-arginyl-glycyl-APP methioninol,

tyrosyl-D-alanyl-glycyl-homophenylalanyl-methionine amide,

tyrosyl-D-alanyl-glycyl-homophenylalanyl methioninol,

tyrosyl-D-arginyl-glycyl-homophenylalanyl-methionine

30 amide,

tyrosyl-D-arginyl-glycyl-homophenylalanyl methioninol,

tyrosyl-D-alanyl-glycyl-AHPPA amide,

tyrosyl-D-arginyl-glycyl-AHPPA amide,

tyrosyl-D-alanyl-glycyl-APP amide,

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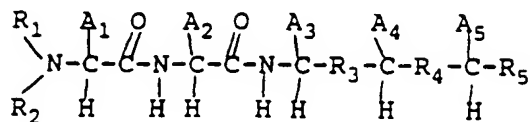
- tyrosyl-D-arginyl-glycyl-APP amide,
tyrosyl-D-alanyl-glycyl-homophenylalanine amide,
tyrosyl-D-arginyl-glycyl-homophenylalanine amide,
tyrosyl-D-alanyl-DOPA-AHPPA amide
- 5 tyrosyl-D-arginyl-DOPA-AHPPA amide
tyrosyl-D-alanyl-DOPA-APP amide
tyrosyl-D-arginyl-DOPA-APP amide
tyrosyl-D-alanyl-DOPA-homophenylalanine amide,
tyrosyl-D-arginyl-DOPA-homophenylalanine amide,
- 10 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-AHPPA amide,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-AHPPA amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-APP amide,
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-APP amide,
tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-
- 15 homophenylalanine amide, or
tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-
homophenylalanine amide.

12. The peptide of claim 11 having the formula
of:

- 20 tyrosyl-D-alanyl-glycyl-APP-methionine amide,
tyrosyl-D-arginyl-glycyl-APP-methionine amide,
tyrosyl-D-alanyl-glycyl-homophenylalanyl-methionine
amide,
tyrosyl-D-arginyl-glycyl-homophenylalanyl-methionine
- 25 amide,
tyrosyl-D-alanyl-glycyl-AHPPA amide,
tyrosyl-D-arginyl-glycyl-AHPPA amide,
tyrosyl-D-arginyl-glycyl-APP amide.

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13. A peptide of the formula:



5 in which

A_1 is the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-dimethyltyrosine;

10 A_2 is the identifying group of an amino acid selected from D-Ala and D-Arg;

A_3 is H or the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine and 3,4-dimethoxyphenylalanine;

15 A_4 is H, cyclohexylmethyl, the identifying group of an amino acid selected from 3,4-dihydroxyphenylalanine, 3,4-dimethoxyphenylalanine, Phe, and substituted Phe with its benzene ring substituted by halogen, NO_2 , OH, or CH_3 ;

20 A_5 is the identifying group of a D- or L-amino acid selected from Leu, Nle, Lys, Met and Met(O), or is deleted together with R_4 -CH attached thereto;

each R_1 and R_2 is $-H$, $-C(NH_2)=NH$, or C_{1-12} alkyl;

R_3 is $-CH_2NH-$ or $-CO \cdot NH-$;

25 R_4 is $-CH_2NH-$ or $-CO \cdot NH-$;

R_5 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n \overset{O}{\underset{||}{C}}NXH$, or $-(CH_2)_n \overset{S}{\underset{||}{C}}NXH$;

wherein n is 0-6, and X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18} aralkyl, C_{7-18} alkaryl, C_{7-18} alkaryl, C_{6-17} pyridylalkyl, or C_{6-17} alkylpyridyl; provided that one and only one of A_3 and A_4 is H, that when one of R_1 and R_2 is $-C(NH_2)=NH$, the other must be H, and that one and only one of R_3 and R_4 is $-CH_2NH-$; or a pharmaceutically acceptable salt thereof.

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14. The peptide of claim 13, wherein
 A_1 is the identifying group of an amino acid
 selected from 3,4-dihydroxyphenylalanine, 3,4-
 dimethoxyphenylalanine, and Tyr;
- 5 A_5 is the identifying group of a D- or L-amino
 acid selected from Leu, Met and Met(O), or is deleted
 together with R_4 -CH attached thereto;
 each R_1 and R_2 is -H or $-C(NH_2)=NH$;
- 10 R_5 is $-(CH_2)_{n+1}OH$ or $-(CH_2)_n\overset{O}{\parallel}CNXH$;
 n is 0-2; and
 X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.
15. The peptide of claim 14, wherein
 A_4 is the identifying group of an amino acid selected
 15 from 3,4-dihydroxyphenylalanine and 3,4-
 dimethoxyphenylalanine.
16. The peptide of claim 14 having the formula
 of:
- 20 tyrosyl-D-alanyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 ethylamide,
 tyrosyl-D-alanyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 propylamide,
 tyrosyl-D-arginyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 ethylamide,
 25 tyrosyl-D-arginyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 propylamide,
 amidinotyrosyl-D-alanyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 ethylamide,
 amidinotyrosyl-D-alanyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 propylamide,
 30 amidinotyrosyl-D-arginyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 ethylamide,
 amidinotyrosyl-D-arginyl-glycyl- $\psi(CH_2NH)$ -phenylalanine
 propylamide,

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- tyrosyl-D-arginal-glycyl-phenylalanyl- ψ (CH₂NH)-leucine
amide,
- tyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine ethylamide,
tyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine propylamide,
- 5 tyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine ethylamide,
tyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine
propylamide,
- amidinotyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine
ethylamide,
- 10 amidinotyrosyl-D-alanyl-DOPA- ψ (CH₂NH)-phenylalanine
propylamide,
- amidinotyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine
ethylamide,
- amidinotyrosyl-D-arginyl-DOPA- ψ (CH₂NH)-phenylalanine
propylamide,
- 15 tyrosyl-D-arginal-DOPA-phenylalanyl- ψ (CH₂NH)-leucine
amide,
- tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine ethylamide,
- 20 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine propylamide,
- tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine ethylamide,
- tyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl- ψ (CH₂NH)-
phenylalanine propylamide,
- 25 amidinotyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)-phenylalanine ethylamide,
- amidinotyrosyl-D-alanyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)-phenylalanine propylamide,
- 30 amidinotyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)-phenylalanine ethylamide,
- amidinotyrosyl-D-arginyl-3,4-dimethoxyphenylalanyl-
 ψ (CH₂NH)-phenylalanine propylamide, or
- tyrosyl-D-arginal-3,4-dimethoxyphenylalanyl-phenylalanyl-
35 ψ (CH₂NH)-leucine amide.

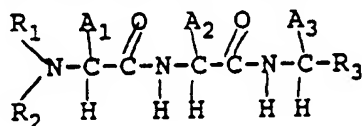
- 40 -

17. The peptide of claim 16 having the formula of:

- tyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
- 5 tyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
- tyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
- 10 tyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
- amidinotyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
- amidinotyrosyl-D-alanyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,
- 15 amidinotyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
ethylamide,
- amidinotyrosyl-D-arginyl-glycyl- ψ (CH₂NH)-phenylalanine
propylamide,.

18. A peptide of the formula:

20



in which

A₁ is the identifying group of an amino acid
25 selected from 3,4-dihydroxyphenylalanine, 3,4-
dimethoxyphenylalanine, azatyrosine, Tyr, and 2,6-
dimethyltyrosine;

A₂ is the identifying group of an amino acid
selected from D-Ala and D-Arg;

30 A₃ is the identifying group of an amino acid
selected from 3,4-dihydroxyphenylalanine and 3,4-
dimethoxyphenylalanine, or is deleted together with CO-
NH-CH attached thereto;

each R₁ and R₂ is -H, -C(NH₂)=NH, or C₁₋₁₂ alkyl;

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R_3 is $-(CH_2)_{n+1}OH$, $-(CH_2)_n\overset{O}{\parallel}CNXH$, or $-(CH_2)_n\overset{S}{\parallel}CNXH$;
 wherein X is H, C_{1-12} alkyl, C_{6-12} aryl, C_{7-18}
 aralkyl, C_{7-18} alkaryl, C_{7-18} 3,4-dihydroxyphenylalkyl, C_{7-18}
 3,4-dimethoxyphenylalkyl, C_{6-17} pyridylalkyl, or C_{6-17}
 alkylpyridyl; or a pharmaceutically acceptable salt
 thereof.

19. The peptide of claim 18, wherein
 A_1 is the identifying group of an amino acid
 selected from 3,4-dihydroxyphenylalanine, 3,4-
 dimethoxyphenylalanine, and Tyr;

each R_1 and R_2 is $-H$ or $-C(NH_2)=NH$;

R_3 is $-(CH_2)_n\overset{O}{\parallel}CNXH$; and
 X is H, C_{1-12} alkyl, or C_{7-18} aralkyl.

20. The peptide of claim 18, wherein
 A_3 is deleted together with $CO-NH-CH$ attached thereto.

21. The peptide of claim 20 having the formula
 of:

tyrosyl-D-alanyl-DOPA amide,
 tyrosyl-D-arginyl-DOPA amide,
 tyrosyl-D-alanyl-3,4-dimethoxyphenylalanine amide,
 tyrosyl-D-arginyl-3,4-dimethoxyphenylalanine amide,
 tyrosyl-D-alanine 3-(3',4'-dihydroxyphenylpropyl)amide,
 tyrosyl-D-arginine 3-(3',4'-dihydroxyphenylpropyl)amide,
 tyrosyl-D-alanine 3-(3',4'-dimethoxyphenylpropyl)amide,
 tyrosyl-D-arginine 3-(3',4'-dimethoxyphenylpropyl)amide,
 DOPA-D-alanine 3-phenylpropylamide,
 DOPA-D-alanine 2-(2-aminoethylpyridyl)amide, or
 DOPA-D-arginine 2-(2-aminoethylpyridyl)amide.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10711

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 37/02; C07K 5/10, 7/06

US CL : 530/330; 514/17,18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/330; 514/17,18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Tetrahedron, Vol.44, No.3, issued 1988, Coy et al, "Solid Phase Reductive Alkylation Techniques in Analogue Peptide Bond and Side-Chain Modification", pages 835-841, see entire document.	1-21
Y	Chem. Pharm. Bull., Vol.39, No.9, issued 1991, Sasaki, et al, "Studies on Analgesic Oligopeptides. VII. Solid Phase Synthesis and Biological Properties of Tyr-D-Arg-Phe-beta Ala-NH ₂ and Its Fluorinated Aromatic Amino Acid Derivatives", pages 2316-2318, see entire document."	1-21

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

31 January 1994

Date of mailing of the international search report

11 FEB 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10711

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Peptides, Vol. 2, issued 1981, Vavrek et al, "Minimum Structure Opioids-Depeptide and Tripeptide Analogs of the Enekephalins", pages 303-308, see entire document.	<u>18-18</u> 1-21

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